

Exhibit 1

Translation of the Sumio reference

* NOTICES *

JPO and INPIT are not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the active oxygen elimination agent in the living body which makes an active principle the sesamin and/or episesamin which have the specific prehension operation over the active oxygen which has a powerful bioactive operation, especially OH radical.

[0002]

[Description of the Prior Art] It is easy to understand the indispensable nature of oxygen sensuously, and, on the other hand, it is also a fact that oxygen has done a failure to the living thing. Producing this oxygen failure, when the singlet oxygen ($1O_2$) which is the superoxide radical which is the reduction molecular species of reactant high oxygen (O_2^-), a hydrogen peroxide (H_2O_2), OH radical ($-OH$), and an excited molecule kind oxidizes a target molecule, they are collectively called active oxygen by these molecular species.

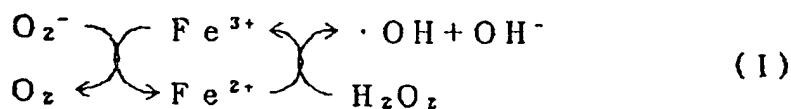
[0003] Moreover, since the operation same also as the unsaturated fatty acid peroxy radical (LOO^-) which is the oxide of a cell component, especially a partial saturation lipid, an unsaturated fatty acid radical (L^-), an unsaturated fatty acid hydroperoxide ($LOOH$), and an unsaturated fatty acid alkoxy radical (LO^-) is shown, it may be called active oxygen also including these. In order to prevent this oxygen failure, first, the living thing kept the amount of generation of active oxygen low, and has prevented oxidation of a target molecule by eliminating the active oxygen generated further. However, when generation control or elimination of active oxygen do not fully function, under the physics which generation of active oxygen increases, and a chemical environmental condition, active oxygen oxidizes a target molecule and various failures and diseases are caused by it.

[0004] It is thought that a superoxide radical with a comparatively long life (O_2^-), a hydrogen peroxide (H_2O_2), and an unsaturated fatty acid hydroperoxide ($LOOH$) are eliminated by oxygen, and the short active oxygen of other lives is eliminated by low molecular weight compounds, such as an ascorbic acid, as an elimination device of active oxygen in the living body. For example, the superoxide radical generated within the erythrocyte (O_2^-) All are eliminated by superoxide DISU mutase (SOD) for almost, a hydrogen peroxide (H_2O_2) is eliminated by a catalase and the peroxidase, and singlet oxygen ($1O_2$) is eliminated by beta carotene and the tocopherol. Moreover, there is not only active oxygen but a thing which acts on elimination of an organic radical including a lipid radical in an elimination low molecular weight compound, therefore it acts also on generation control of active oxygen in many cases.

[0005] however, reactivity is high among active oxygen and a specific elimination low molecular weight compound is now to OH radical by which the biohazard operation is also considered to be the largest -- it is not found out. Moreover, it is thought that OH radical has a short life in order to react at the rate almost near a cell component and a diffusion limitation, and a living thing cannot have the special device which eliminates this.

[0006] When the damage does not do a failure to a living thing of the component which approaches and exists in a target molecule Although it has an elimination operation of OH radical to some extent, a living thing is a superoxide radical (O_2^-) rather. A hydrogen peroxide (H_2O_2) is eliminated as completely as possible. By making transition-metals ion exist furthermore in the form which does not carry out OH radical formation catalyst, generation of OH radical is controlled and it is thought that the oxygen failure is prevented. Therefore, if OH radical occurs by chance, it reacts with the molecule which met first, and since, as for a cell, a failure is received as the molecule is important for the function of a cell, OH radical can also be said to be the true causative agent of the disease by active oxygen.

[0007] generation of this OH radical in the living body -- following reaction-formula (I): -- [Formula 1]



[0008] It is thought that it is based on an iron catalyst harbor bypass reaction from the superoxide radical (O₂⁻) boiled and depended, and although examination of SOD which carries out the catalyst of the disproportionation of a superoxide radical (O₂⁻), or a SOD Mr. active substance was performed variously, when it has a trouble in the field of stability, a direct OH radical is not eliminated, and there is nothing that has still succeeded in commercialization.

[0009]

[Problem(s) to be Solved by the Invention] Therefore, this invention tends to offer the active oxygen elimination agent which has a direct prehension operation to the active oxygen which has a powerful bioactive operation, especially OH radical.

[0010]

[Means for Solving the Problem] In order that this invention person etc. may attain the above-mentioned purpose, as a result of studying many things, a sesame seed, The sesamin and/or episesamin which were obtained by composition or it isolated from the inside of ***** and sesame oil A hydrogen peroxide (H₂O₂), a superoxide radical (O₂⁻), Although a prehension operation of an organic radical and singlet oxygen (1O₂) is not shown at all OH radical is caught specifically. Further After [prehension] sesamin And the decomposition product of episesamin () [2-] (3 and 4-methylenedioxyphenyl)-6- (3 and 4-dihydroxy phenyl)-cis- - 3 and 7-dioxa bicyclo [3, 3, 0] octane and 2-(3, 4-methylenedioxyphenyl)-6 -(3, 4-dihydroxy phenyl)- A transformer -3 and 7-dioxa bicyclo [3, 3, 0] octane Hydrogen peroxide It found out having the prehension ability of (H₂O₂), singlet oxygen (1O₂), a superoxide radical (O₂⁻), OH radical, an organic radical, and all lipid radicals. That is, it carried out [**], without being decomposed into the target tissue in which OH radical exists, and header this invention was completed for the completely ideal fact of being changed into the compound which has all active oxygen prehension ability, after OH radical prehension.

[0011] Therefore, this invention tends to offer the active oxygen elimination agent in the living body which makes an active principle the sesamin and/or episesamin which have the specific prehension operation over OH radical.

[0012]

[Specific Explanation] The sesamin and episesamin which are used in this invention are contained about 0.5% in sesame oil, and are rich in practicality in respect of supply, and, moreover, its safety is high. The sesamin and episesamin which are used by this invention are independent, or can mix and use these. Moreover, the extract containing sesamin and/or episesamin may be used.

[0013] The following procedure can perform in the sesamin and the episesamin list which are the active principle of this invention as an approach of obtaining the extract which uses this compound as a principal component. First, in order to obtain the extract which uses as a principal component the compound which is the active principle of this invention from sesame oil, it is obtained by an extract and condensing using the various organic solvents which can extract and dissolve the compound which sesame oil is nonmiscible substantially and is the active principle of this invention. As such an organic solvent, an acetone, a methyl ethyl ketone, a diethyl ketone, a methanol, ethanol, etc. can be mentioned.

[0014] After mixing sesame oil and either of the above-mentioned solvents to homogeneity in order to obtain the extract which uses as a principal component the compound which is the active principle of this invention for example, it puts in low temperature, phase separation is performed according to conventional methods, such as centrifugal separation, and it is obtained by carrying out evaporation removal of the solvent from a solvent fraction. Two to 10 times, sesame oil is preferably melted to the acetone of capacity six to 8 times, and is still more specifically left at -80 degrees C overnight. As a result, an oil component is precipitating, an organic solvent is distilled out of the filtrate obtained by filtration, and the extract which uses this invention compound as a principal component is obtained. Or after mixing sesame oil by the heat methanol or heat ethanol, it puts in a room temperature and is obtained by carrying out evaporation removal of the solvent from a solvent fraction.

[0015] Two to 10 times, it mixes five to 7 times preferably by the heat methanol (50 degrees C or more) or heat ethanol (50 degrees C or more) of capacity, and sesame oil is still more specifically extracted violently. According to conventional methods, such as standing or centrifugal separation, phase separation is carried out to a room temperature, a solvent is distilled out of a solvent fraction, and the extract which uses this invention compound as a principal

0023] For example, when preparing injections, the solubilizing agent for drugs, such as a nonionic surface active agent, can be used, nonionic surface active agents, such as POE (60) hydrogenated castor oil of 80 time capacity or POE sorbitan mono-olate, can be made to be still more specifically able to carry out the heating dissolution of the

compound of this invention, and it can prepare by diluting with a physiological saline. Moreover, an isotonizing agent, a stabilizer, antiseptics, and an aponia-ized agent may be added suitably if needed. Moreover, as external preparations, an ointment, cream pharmaceuticals, etc. can be prepared by the usual approach, using vaseline, paraffin, fats and oils, lanolin, macro gall, etc. as a basis.

[0024] Although the gestalt of the above-mentioned pharmaceutical preparation is sufficient when using the compound of this invention as an eating-and-drinking article, this invention compound of requirements can be added to a food raw material, and processing manufacture can be carried out according to a general manufacturing method. Under the present circumstances, the class of food and especially a gestalt are not limited. Moreover, since the intake as health food and functional food is used for sick prevention and health maintenance, it is desirable to be taken in as a workpiece which contains a day in 1-100mg /as an ingestion. Moreover, vitamin C, vitamin E, beta carotene, SOD, etc. can be used together with other active oxygen elimination agents. As for especially the compound that has antioxidation nature, such as vitamin C and vitamin E, it is useful to also have the operation as a stabilizing agent of this invention compound, and to use together, and it can be expected that vitamin E will reinforce the effectiveness of this invention compound.

[0025] Conventionally, since the compound which is the active principle of this invention is a compound found out from the inside of food, excel [from the field of safety] is clear. When this carries out pitching in successive games (internal use) of sesamin 2.14 g/day / kg for two weeks to the ICR male mouse of 7 weeks old again, the symptom unusual in any way is clear also from having not accepted, either.

[0026]

[Example] Next, an example explains this invention still more concretely.

[0027] Example 1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction activity Brois An approach (M. S.Brois, Nature, 181, and 1199-1200 (1958)) is followed. 25micro[of the sesamin / episesamin (6:4) mixture which 2ml of ethanol solutions of 0.1mMDPPH(s) was made to dissolve in dimethyl sulfoxide (DMSO) by predetermined concentration (dilution sequence), or the alpha-tocopherol (as a contrast compound)] l is added. The absorbance in 516nm was measured after 20 minutes. This reduction activity is seeing reduction in an absorbance as an index of the elimination activity of a DPPH radical, and this activity is not accepted in sesamin / episesamin (6:4) mixture so that clearly from drawing 1 . The addition concentration shown in drawing 1 shows the last concentration.

[0028] Example 2. Superoxide elimination activity phenazine meso sulfate (0.6mM) 5microl, Nitroblue tetrazolium (1mM) 50microl, the sesamin / episesamin (6:4) mixture which dimethyl sulfoxide (DMSO) was made to dissolve in the mixed liquor of 370micro of phosphate buffers l (0.1M, pH7.5) by predetermined concentration (dilution sequence), the alpha-tocopherol, Or after adding 25microl of a quercetin (all are a contrast compound), Added NADH(1.6mM) 50microl, the reaction was made to start, and the inhibition (elimination activity) to the increment in the absorbance (560nm) accompanying the nitroblue tetrazolium reduction by the superoxide produced in this system of reaction was seen. It asked for the rate of inhibition (%) from $x(1-a/A)$ 100 when setting absorbance augend per unit time amount at the time of adding A and a compound for the absorbance augend per unit time amount in a blank (only solvent) to a. As shown in drawing 2 , superoxide elimination activity was not accepted in sesamin / episesamin (6:4) mixture. The addition concentration shown in drawing 2 shows the last concentration.

[0029] Example 3. The system of reaction used by OH radical prehension activity this example by the deoxyribose method generates OH radical at the Fenton reaction, and is based on the approach of measuring the thiobarbituric acid-MDA adduct generated when the malondialdehyde (MDA) produced by the reaction of the OH radical and deoxyribose is made to react with thiobarbituric acid.

[0030] 0.1M [namely,] Into the mixed liquor which consists of deoxyribose (1.43mM) 690microl, and FeSO₄ / EDTA mixture (each 1mM) 10microl dissolved in the phosphate buffer (pH7.4) The sesamin / episesamin (6:4) mixture dissolved in dimethyl sulfoxide (DMSO) by predetermined concentration (dilution sequence), Or 100micro[of the alpha-tocopherol (as a contrast compound)] l is added, and it is 0.1M. H₂ O₂ 200 (5mM)microl dissolved in the phosphate buffer (pH7.4) is added, and it is made to react at 28 degrees C for 16 hours.

[0031] After a reaction, 0.5ml (2.8%) of trichloroacetic acids, 50mM Add 0.5ml (1%) of thiobarbituric acid dissolved in NaOH, and it was made to boil for 10 minutes, and after making this cool, the absorbance of 535nm was measured. It asked for the rate of inhibition (%) from $x(1-a/A)$ 100 when setting the absorbance at the time of adding A and a compound for the absorbance in a blank (only solvent) to a. As shown in drawing 3 , I50 value which OH radical prehension activity strong against sesamin / episesamin (6:4) mixture is accepted, and does inhibition 50% was about

3microM. The addition concentration shown in drawing 3 shows the last concentration.

[0032]

Example 4. The system of reaction used by microsome lipid-peroxidation control activity this example generates OH radical by NADPH in a rat liver microsome, and is based on the approach of measuring the thiobarbituric acid-MDA adduct generated when the malondialdehyde (MDA) produced in the process in which the OH radical peroxidates a microsome film lipid is made to react with thiobarbituric acid.

[0033] That is, after adding 5micro[of the sesamin dissolved in dimethyl sulfoxide (DMSO) by predetermined concentration (dilution sequence), episesamin, or the alpha-tocopherol (as a contrast compound)] 1 and making it dissolve, rat liver microsome (1mg protein / ml) 0.5ml which 0.1M phosphate buffer (pH7.4) was made to suspend is added to 0.5ml (pH7.4) of 0.1M phosphate buffers, and the ink bait of the 37 degrees C is carried out to them for 5 minutes. A reaction is Buege in order to start by adding NADPH(1.5mM)0.25ml dissolved in 0.1M phosphate buffer (pH7.4), to make 37 degrees C react for 20 minutes and to stop a reaction. The thiobarbituric acid reagent based on the approach (J. A.Buege and S.T.D.Aust, Meth.Enzymol., 52,302-310, (1978)) of Aust was added.

[0034] Then, the absorbance of 535nm after carrying out centrifugal removal of the precipitate which is made to boil this for 10 minutes and is produced was measured. It asked for the rate of inhibition (%) from $x(1-a/A) 100$ when setting the absorbance at the time of adding A and a compound for the absorbance in a blank (only solvent) to a. As shown in drawing 4, sesamin and the episesamin of 150 value which microsome film lipid-peroxidation control activity strong against sesamin and episesamin is accepted, and does inhibition 50% were about 0.3microM. The addition concentration shown in drawing 4 shows the last concentration.

[0035] Example 5. OH radical decomposition product of 1 of sesamin and episesamin decomposition product and 1-diphenyl-2-picryl hydrazine (DPPH) reduction activity sesamin and episesamin was prepared. 0.1M 0.1M after the sesamin melted to the acetone or 1ml of episesamin (10mg/(ml)) having added to 40ml (pH7.4) of phosphate buffers and making it dissolve in them Rat liver microsome (3.99mg protein / ml) 50ml which the phosphate buffer (pH7.4) was made to suspend was added, and 37 degrees C was incubated for 10 minutes. A reaction is 0.1M. It started by adding NADPH(60mM)10ml dissolved in the phosphate buffer (pH7.4).

[0036] It is made to react for 2 hours and 37 degrees C of chloroform are used. Sesamin, episesamin, A sesamin decomposition product and an episesamin decomposition product are extracted from a reaction solution, and it is an opposition column (ODS-50 (Asahipak) is used). Follow a conventional method, and high performance chromatography isolates and refines. 2- (3 and 4-methylenedioxyphenyl)-6- (3 and 4-dihydroxy phenyl)-cis- - 3 and 7-dioxa bicyclo [3, 3, 0] octane (compound A) and 2-(3, 4-methylenedioxyphenyl)-6 -(3, 4-SHIHIDOROKISHI phenyl)-A transformer -3, 7-dioxa bicyclo [3, 3, 0] octane (Compound B) was identified. DPPH reduction ability was measured according to the example 1. 0.1mM(s) 75microl addition of the compound A or B (concentration amM) melted in 2ml of ethanol solutions of DPPH at dimethyl sulfoxide (DMSO) was done, and the absorbance in 516nm was measured after 20 minutes. It asked for compound A or the DPPH reduction ability of B by the degree type.

[0037]

[Equation 1]

$$\frac{\frac{y-x}{y} \times A \times D}{a} = \text{mM} / \text{mM equ}$$

[0038] x is an absorbance in 516nm obtained by compound A or B addition. y is an absorbance in 516nm obtained at the time of compound additive-free. A is the DPPH concentration (0.1) shown by mM. D is the compound A in reaction mixture, or the dilution ratio (2025/25) of B solution. a is the concentration shown by mM of compound A or B. The result of having measured the DPPH reduction ability of compound A, B, sesamin, or episesamin is shown in Table 1.

[0039]

表1

サンプル	D P P H還元能 (mM/mEq)
化合物A	0.75
化合物B	0.72
セサミン	0
エピセサミン	0

[0040] Furthermore, when microsome lipid-peroxidation control activity was measured according to the example 4, as shown in drawing 5, compound A and B showed sesamin and the activity more than equivalent. As mentioned above, the sesamin and episesamin which are OH radical alternative elimination agent became clear [carrying out prehension elimination also to active oxygen, such as an organic radical and a superoxide radical, and a free radical], when a metabolic turnover was received in a target tissue in the living body.

[0041] 2.4g of this invention compounds was added to 100g of butterfats from which buttermilk was removed by **** actuation (churning) of an example 6. butter making process, and this invention compound content butter was obtained as a presentation with an equal deed of **** actuation (working).

[0042] 0.5g of example 7. this invention compounds was mixed with 20.5g of silicic acid anhydrides, and corn starch 79g was added to this, and it mixed further. 100ml of hide ROKISHI propyl cellulose ethanol solutions was added to this mixture 10%, kneading was carried out as the conventional method, and it extruded, and it dried and the granule was obtained.

[0043] 7g of example 8. this invention compounds was mixed with 20g of silicic acid anhydrides, and microcrystal cellulose 10g, 3.0g of magnesium stearates, and 60g of lactoses were added to this, it mixed, this mixture was tableted with the single-engined type tableting machine, and 7mm of diameters and a tablet with a weight of 100mg were manufactured.

[0044] The heating dissolution of the 2.5g of the example 9. this invention compounds was carried out at 122 degrees C at TO-10M (Nikko Chemicals)200g which is a nonionic surface active agent, 4.7975l. of sterilization physiological salines warmed at 60 degrees C at this could be added, and it stirred, and this was distributed to the vial in sterile, was sealed, and injections were manufactured.

[0045] Propylene glycol 7g is added and heated to 53g of example 10. purified water, and it keeps at 70 degrees C (aqueous phase). 2g [of this invention compounds] and microcrystalline wax 1g, yellow-bees-wax 2g, 18g [of liquid paraffins], and squalene 10g, 4g of sorbitan sesquioleate, and 1g of polyoxyethylene (20 mols) SORIBI tongue mono-oleate are mixed, and after heating to 122 degrees C and dissolving completely, it keeps at 70 degrees C, and the perfume of optimum dose and antiseptics are added and it mixes (oil phase). Stirring an oil phase, the aqueous phase was gradually added to this, it emulsified to homogeneity by the homomixer, and the milky lotion was manufactured by cooling to 30 degrees C with after [emulsification] stirring.

[0046] 0.5g of example 11. this invention compounds and 0.5g of vitamin C were mixed with 20g of silicic acid anhydrides, and corn starch 79g was added to this, and it mixed further. 100ml of hide ROKISHI propyl cellulose ethanol solutions was added to this mixture 10%, kneading was carried out as the conventional method, and it extruded, and it dried and the granule was obtained.

[0047] 5g of example 12. this invention compounds and 2g of tocopherol acetate were mixed with 20g of silicic acid anhydrides, and microcrystal cellulose 10g, 3g of magnesium stearates, and 60g of lactoses were added to this, it mixed, this mixture was tableted with the single-engined type tableting machine, and 7mm of diameters and a tablet with a weight of 100mg were manufactured.

[0048]

[Effect of the Invention] The active oxygen elimination agent of this invention which consists of the result of an example including sesamin and/or episesamin An ischemia reperfusion failure (organ derangement, such as a myocardiopathy, a gastric-mucosa failure, and liver), inflammation (blood vessel permeability sthenia) a vascular endothelial cell failure, production of Peroxidation LDL, and a digestive system disease (stress --) A shock, an ischemic

organization failure, a kidney disease (methylguanidine production, such as ischemic acute renal failure and glomerulonephritis), Reactivity is the highest also in the active oxygen which is the causes, such as an endocrinologic disease and a cataract, (diabetes mellitus etc.). It catches and eliminates specifically to OH radical without the defense mechanism in a living body. After sesamin and /episesamin receive a metabolic turnover in a target tissue in the living body, it is clear that this invention is very more useful than the decomposition product has prehension / elimination ability also to active oxygen, such as an organic radical and a superoxide radical, and a free radical.